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ACUTE DERMAL IRRITATION STUDY OF TEN JET FUELS IN NEW ZEALAND WHITE RABBITS: COMPARISON OF SYNTHETIC AND BIO-BASED JET FUELS WITH PETROLEUM JP-8

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The experiments reported were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

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14. ABSTRACT

The dermal irritation potential of ten jet fuels of interest to the U.S. Air Force was studied, including petroleum-derived JP-8. Five of the alternative fuels were synthetic paraffinic kerosenes (SPK) derived from non-petroleum fossil fuel feedstocks utilizing a Fischer-Tropsch synthesis (FT-SPK): Sasol iso-paraffinic kerosene (IPK), Sasol gas to liquid (GTL)-1 and GTL-2, Shell GTL and Syntroleum S-8 (synthetic JP-8). Four fuels were renewable bio-based fuels: Syntroleum R-8 hydroprocessed esters and fatty acids jet fuel derived from mixed fats and oils (HEFA-F), Syntroleum Sapphire derived from algae (HEFA-A), Amyris IPK and Swedish Biofuel. Each fuel was applied for four hours to the skin of New Zealand White (NZW) rabbits using occluded or semi-occluded exposures, and evaluated by the Draize method. A score of slightly irritating was determined for the occluded and semi-occluded exposures to all test substances, with the exception of a score of non-irritating for the semi-occluded exposures of Sasol IPK, Syntroleum S-8 and HEFA-F. Very slight erythema persisted through study day 14 for the occluded exposures to Amyris IPK, Swedish Biofuel, and HEFA-A. Normal handling of these fuels should not result in increased dermal irritation among airmen.

15. SUBJECT TERMS
Dermal irritation, jet fuels, alternative fuels, synthetic paraffinic kerosene, JP-8, biobased/bio-based, toxicity/toxicology

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PREFACE

Funding for this project was provided through the Alternative Fuels Certification Office (AFLCMC/WNN). This research was conducted under contract FA8650-10-2-6062 with the Henry M. Jackson Foundation for the Advancement of Military Medicine (HJF). The program manager for the HJF contract was David R. Mattie, PhD (711 HPW/RHPB, now 711 HPW/RHDJ), who was also the technical manager for this project.

The dermal irritation study protocol was designed to be in general compliance with the U.S. Environmental Protection Agency (EPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) Guideline 870.2500 (1998) and the Organisation for Economic Co-operation and Development (OECD) Guidelines for Testing of Chemicals, Section 404 (2002). The study was conducted in compliance with 40 CFR Part 792, Good Laboratory Practice Standards (GLP).

The study protocol was approved by the Air Force Surgeon General's Office of Research Oversight and Compliance (protocol number FWR-2010-0001A, Acute Dermal Irritation Study of Alternative and Bio-Based Jet Fuels in New Zealand White Rabbits, *Oryctolagus cuniculus*) and the WIL Research Laboratories, LLC, Animal Care and Use Committee (protocol number WIL-773001). The study was conducted in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International, in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 2011).

The authors would like to acknowledge J. Tim Edwards, Ph.D. (Air Force Research Laboratory Fuels Branch, AFRL/RQTF, Wright-Patterson AFB OH) for his help identifying and describing the fuels in this study.

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1.0 SUMMARY

The objective of this study was to determine the dermal irritation potential of ten jet fuels of interest to the U.S. Air Force, including petroleum-derived JP-8 (POSF 4658) and nine alternative jet fuel candidates. All fuels were identified by a commercial name and by the POSF log book number provided by the Air Force Research Laboratory Fuels Branch (AFRL/RQTF, Wright-Patterson AFB OH). All fuels contained the standard JP-8 additive package.

Five of the alternative fuels were synthetic paraffinic kerosenes (SPK) derived from non-petroleum fossil fuel feedstocks utilizing a Fischer-Tropsch synthesis (FT-SPK):

- Sasol iso-paraffinic kerosene (IPK, Sasol Synfuels International (SSI), Johannesburg, South Africa, POSF 5642);
- Sasol gas to liquid (GTL)-1 (POSF 5976) and GTL-2 (POSF 5977);
- Shell GTL (POSF 5172, Shell Global, The Hague, The Netherlands); and
- Syntroleum S-8 (synthetic JP-8, POSF 4734, Syntroleum Corporation, Tulsa OK).

Four fuels were renewable bio-based fuels:

- Syntroleum R-8 hydroprocessed esters and fatty acids (HEFA) jet fuel derived from mixed fats and oils (HEFA-F, POSF 5469);
- Syntroleum Sapphire derived from algae (HEFA-A, POSF 5804);
- Amyris IPK (Amyris, Inc., Emeryville CA, POSF 5630); and
- Swedish Biofuel (Swedish Biofuels AB, Stockholm, Sweden, POSF 5668).

Each fuel was applied to the skin of New Zealand White rabbits. Doses (0.5 mL) of each fuel were applied to separate areas of clipped, unabraded skin (six application sites/rabbit, six rabbits per group, two groups (occluded or semi-occluded)). After four hours of exposure, the bandages were removed and the sites washed. Application sites were evaluated in accordance with the method of Draize (1965) and in compliance with the U.S. Environmental Protection Agency (EPA, 1998) and Organisation for Economic Co-operation and Development (OECD, 2002) at approximately 30 to 60 minutes and 24, 48 and 72 hours after patch removal, and on study days 4, 7 and 14, if irritation persisted.

There were no deaths or remarkable body weight changes noted during the study. Dermal findings during the study consisted of very slight to slight erythema, as well as very slight edema. A score of slightly irritating, as evaluated by the Primary Dermal Irritation Index (PDII) and Descriptive Rating, was calculated for the occluded and semi-occluded exposures to all test substances, with the exception of a score of non-irritating for the semi-occluded exposures of Sasol IPK, Syntroleum S-8 and HEFA-F. Very slight erythema persisted through study day 14 for the occluded exposures to Amyris IPK, Swedish Biofuel, and HEFA-A. It is not expected that normal handling of these fuels would result in increased dermal irritation among airmen.

2.0 INTRODUCTION

Domestically produced alternative fuels for military use are being pursued in order to decrease dependence on foreign oil sources (Blackwell, 2007). These alternative fuels formulated to be used in combination with or in place of petroleum-derived JP-8, the traditional military fuel. As each alternative fuel's composition is different from JP-8, potential health effects from fuel exposure must be considered during fuel development.

Multiple jet fuels have been tested for dermal irritation. In this assay, each rabbit serves as its own control as there are up to six sites per rabbit on which to apply individual fuels; one of the sites is used as a non-treated control. Jet fuel dermal irritation assays are performed using two sets of rabbits; the application sites are occluded in one set and semi-occluded in the second set. JP-8 ranges from slightly to moderately irritating when evaluated using this assay (Hurley *et al.*, 2011; Mattie *et al.*, 2013).

The purpose of this report was to investigate ten jet fuels for dermal irritation potential. All fuels were identified not only by a commercial name but also by the POSF log book number maintained by the Air Force Research Laboratory Fuels Branch (AFRL/RQTF, Wright-Patterson AFB OH). Use of commercial names and products does not constitute endorsement of these products by the U.S. Air Force but does reflect the variety of jet fuels to which airmen may be exposed, now or in the future. All fuels contained the standard JP-8 additive package required by the U.S. Air Force.

The first fuel evaluated was petroleum-derived JP-8 (POSF 4658), included as a "positive" control with which to compare dermal responses from other fuels. Five of the alternative fuels were synthetic paraffinic kerosenes (SPK) derived from non-petroleum fossil fuel feedstocks utilizing a Fischer-Tropsch synthesis process (FT-SPK) (Moses, 2008). Sasol Synfuels International (SSI, Johannesburg, South Africa) developed three SPK fuels evaluated herein; one iso-paraffinic kerosene (IPK, Sasol IPK, POSF 5642) derived from a coal feedstock and two gas to liquid (GTL) formulations (Sasol GTL-1 and GTL-2, POSFs 5976 and 5977, respectively) derived from natural gas. The remaining two SPK fuels, Shell GTL (POSF 5172, Shell Global, The Hague, The Netherlands) and Syntroleum S-8 (synthetic JP-8, POSF 4734, Syntroleum Corporation, Tulsa OK) were also derived from natural gas sources.

The remaining four fuels evaluated were renewable bio-based fuels. One process for converting biomass using hydro-treatment (water and high pressure) results in a fuel known as hydroprocessed esters and fatty acids (HEFA) jet fuel, formerly called hydrotreated renewable jet (HRJ) fuel (Edwards *et al.*, 2012). Two Syntroleum HEFA products were tested in this study: Syntroleum R-8 (renewable JP-8) derived from a feedstock of mixed fats and oils (HEFA-F, POSF 5469) and Syntroleum Sapphire derived from photosynthetic microorganisms (algae, HEFA-A, POSF 5804). Amyris, Inc. (Emeryville CA) developed an IPK from hydroprocessed fermented sugars that was also evaluated in this study (Amyris IPK, POSF 5630). Finally, through an international agreement with Sweden, an alcohol to jet (ATJ) biofuel processed from ethanol to produce normal alkanes, aromatics and cycloparaffins in the jet fuel range was procured for dermal irritation testing (Swedish Biofuel, Swedish Biofuels AB, Stockholm, Sweden, POSF 5668).

3.1 METHODS

The complete study protocol is found in Appendix A.

3.2 Test Substances Identification

Test substances were received from 711 HPW/RHPB (currently 711 HPW/RHDJ), Wright-Patterson Air Force Base (AFB) OH, on 4 May 2010. Table 1 contains the test substances identification and description. Test substances are identified by POSF log book numbers as well as WIL identification numbers (Table 1). Purity and stability data are maintained by the AFRL Fuels Branch.

The test substances were stored at room temperature in a flame cabinet and were considered stable under these conditions. Prior to use, the original container of each test substance was inverted or swirled to ensure a homogeneous mixture. A reserve sample of each test substance was collected and stored in the WIL Archives.

Table 1. Test Substance Identification and Description

Test Substance	Identification	POSF	WIL ID #	Physical Description
1	JP-8 (Jet A Blend)	4658	100096	Clear, very light yellow liquid
2	Sasol IPK	5642	100097	Clear, colorless liquid
3	Sasol GTL-1	5976	100098	Clear, colorless liquid
4	Sasol GTL-2	5977	100099	Clear, colorless liquid
5	Shell GTL	5172	10009A	Clear, colorless liquid
6	Syntroleum S-8	4734	10009B	Clear, colorless liquid
7	HEFA-F (Syntroleum R-8)	5469	10009C	Clear, colorless liquid
8	Amyris IPK	5630	10009D	Clear, colorless liquid
9	Swedish Biofuel	5668	10009E	Clear, colorless liquid
10	HEFA-A (Syntroleum Sapphire)	5804	10009F	Clear, colorless liquid

Notes: POSF: AFRL Fuels Branch log book numbers; WIL ID #: identification numbers maintained by WIL Research Laboratories, LLC

3.3 Animals and Handling

Male New Zealand White albino rabbits were used as the test system on this study. The animal model, the New Zealand White albino rabbit, is generally recognized as appropriate for acute dermal irritation studies. The animals were approximately 23 weeks old at the initiation of dose administration.

New Zealand White albino rabbits utilized for this study were received in good health from Covance Research Products, Inc. (Kalamazoo MI). The rabbits were inspected by a qualified technician upon receipt, weighed and uniquely identified by a plastic ear tag displaying the animal number. The rabbits were acclimated to laboratory conditions for a minimum of five days. During this period, each animal was observed twice daily for changes in general appearance or behavior.

Upon arrival, all animals were housed in individual stainless steel cages. The animals were maintained by the animal husbandry staff of WIL in accordance with standard operating procedures (SOPs). The animal facilities at WIL are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

The basal diet used in this study, PMI Nutrition International, LLC (St. Louis MO) Certified Rabbit HF LabDiet® 5325, is a certified feed with appropriate analyses performed by the manufacturer and provided to WIL. Municipal water supplying the facility was analyzed for contaminants according to WIL SOPs. The results of the diet and water analyses are maintained at WIL. No contaminants were present in animal feed or water at concentrations sufficient to interfere with the objectives of this study. The basal diet was provided at approximately 150 g/day while municipal water, delivered by an automatic watering system, was provided *ad libitum* throughout the acclimation period and during the study.

All animals were housed throughout the acclimation period and during the study in an environmentally controlled room. The room temperature and humidity controls were set to maintain environmental conditions of 66 ± 5°F (19 ± 3°C) and 50 ± 20 percent relative humidity. Room temperature and relative humidity data were recorded approximately hourly. Fluorescent lighting provided illumination for a 12 hour light (0600 hours to 1800 hours) photoperiod, followed by a 12 hour dark period. Air handling units were set to provide a minimum of 10 fresh air changes per hour.

3.4 Administration

The selected route of administration for this study was direct application to clipped, unabraded skin (dermal). This route is standard for assessment of local dermal irritative potential. This study was intended to provide information on the health hazards likely to arise from a short-term exposure to the test substances by the dermal route. The experimental design used the procedures and standards in compliance with the U.S. Environmental Protection Agency (EPA, 1998) and the Organisation for Economic Co-operation and Development (OECD, 2002).

Animals used in the study were arbitrarily selected from available stock based upon health and body weight. The selected animals were young adult male rabbits.

There were four groups of three rabbits, five unabraded sites per rabbit (from six available test sites). Animals in Groups 1 and 3 received a single occluded exposure. Animals in Groups 2 and 4 received a single semi-occluded exposure. Table 2 presents the study group assignment.

Table 2. Study Group Assignment

Group Number	Test Substance	Dose Volume (mL)	Exposure Method	Number of Animals
1	1, 2, 3, 4, 5	0.5	Occluded	3
2	1, 2, 3, 4, 5	0.5	Semi-occluded	3
3	6, 7, 8, 9, 10	0.5	Occluded	3
4	6, 7, 8, 9, 10	0.5	Semi-occluded	3

On the day prior to dosing, the hair was removed from the backs and flanks of the rabbits using an electric clipper. Six application sites were available for dosing, as depicted in Figure 1. Fuels were assigned a site in rotation (first fuel applied to site A on first rabbit, B on second rabbit, etc.). Each 0.5-mL dose was applied to an area of skin approximately 2.5 cm × 2.5 cm under a two-ply gauze patch secured in place with Micropore™ tape (3M, St. Paul MN). For animals in the occluded exposure groups, the trunk of the animal was wrapped with plastic wrap to occlude the test site. The trunk of animals in both the occluded and semi-occluded groups was then wrapped with a gauze binder secured with Dermiform® tape (Johnson and Johnson, New Brunswick NJ). Plastic restraint collars were applied to the animals to prevent ingestion of the test substance and/or bandages. After four hours of exposure (actual exposure times ranging from approximately 4 hours 1 minute to 4 hours 26 minutes), the collars and bandages were removed and each of the sites was wiped with a new disposable paper towel moistened with deionized water.

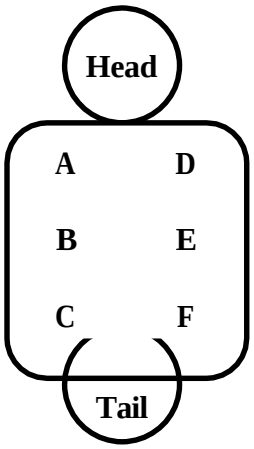

Figure 1. Dermal Dosing Sites on New Zealand White Rabbits

3.5 Observations

The rabbits were observed twice daily, once in the morning and once in the afternoon, for mortality and morbidity. All animals received detailed physical examinations on the day of

dosing. Body weights were obtained and recorded on study day 0 (initiation) and at each rabbit's termination from the study.

The application sites were observed for erythema, edema and other dermal findings approximately 30 to 60 minutes and 24, 48 and 72 hours after patch removal, and, if irritation persisted, on study days 4, 7 and 14. Dermal irritation was graded in accordance with the method of Draize (1965), as detailed in Appendix B. The areas of application were clipped free of hair a minimum of one hour prior to scoring, as needed during the study, to facilitate accurate dermal observations.

The Primary Dermal Irritation Index (PDII) was calculated from scores recorded at 30 to 60 minutes and at 24, 48 and 72 hours after patch removal. The mean scores for erythema and edema were calculated separately to the nearest tenth and added together. Based on this value, the grading system in Appendix B was used to arrive at the PDII descriptive rating.

All data were recorded and reported utilizing WIL in-house data acquisitions and reporting systems detailed in Appendix C. Following study termination, the rabbits were euthanized in accordance with current American Veterinary Medical Association (AVMA) guidelines (AVMA, 2013).

4.1 RESULTS

4.2 General Conditions and Observations

Animal room conditions are summarized in Appendix D. Actual mean daily temperature ranged from 66.0°F to 67.2°F (18.9°C to 19.6°C) and mean daily relative humidity ranged from 50.2 to 55.4 percent during the study.

There were no deaths during the study. Body weight values ranged from 2691 g to 2911 g. No remarkable body weight changes were noted during the study. Individual body weights are detailed in Table 3.

Table 3. Individual Body Weights

Group Number	Test Substance	Animal Number	Day 0 Weight (g)	Termination Weight (g)	Termination Day (Study Day)
1 Occluded	1, 2, 3, 4, 5	60640	2758	2716	4
		60638	2798	3006	14
		60639	2781	3017	14
2 Semi-occluded	1, 2, 3, 4, 5	60636	2773	2838	3
		60634	2878	3012	7
		60637	2737	2875	7
3 Occluded	6, 7, 8, 9, 10	60630	2752	2753	3
		60632	2748	2858	14
		60633	2691	2960	14
4 Semi-occluded	6, 7, 8, 9, 10	60629	2911	2847	3
		60625	2838	3106	14
		60626	2774	2997	14

4.2 Dermal Observations

Dermal findings consisted of very slight (grade 1) to slight (grade 2) erythema for occluded and semi-occluded exposures to all test substances with the exception of the semi-occluded exposures to Sasol/IPK, Syntroleum S-8 and HEFA-F, for which no irritation was noted. Very slight (grade 1) edema was noted for the occluded exposure to Amyris and semi occluded exposure to Swedish Biofuel. Very slight erythema persisted through study day 14 at sites for a single animal for the occluded exposures to Amyris, Swedish Biofuel and HEFA-A. A summary of these results is found in Table 4. Individual score data are found in Appendix E. Application site assignments for each fuel/rabbit are also found in Appendix E.

Table 4. Summary Table of PDII and Descriptive Rating for Dermal Irritation

Test Substance	Identification	Exposure	PDII	Descriptive Rating
1	JP-8	Occluded	0.3	Slightly Irritating
		Semi-occluded	0.6	Slightly Irritating
2	Sasol IPK	Occluded	0.3	Slightly Irritating
		Semi-occluded	0.0	Nonirritating
3	Sasol GTL-1	Occluded	0.3	Slightly Irritating
		Semi-occluded	0.3	Slightly Irritating
4	Sasol GTL-2	Occluded	0.1	Slightly Irritating
		Semi-occluded	0.1	Slightly Irritating
5	Shell GTL	Occluded	0.2	Slightly Irritating
		Semi-occluded	0.2	Slightly Irritating
6	Syntroleum S-8	Occluded	0.3	Slightly Irritating
		Semi-occluded	0.0	Nonirritating
7	HEFA-F (Syntroleum R-8)	Occluded	0.3	Slightly Irritating
		Semi-occluded	0.0	Nonirritating
8	Amyris IPK	Occluded	0.7	Slightly Irritating
		Semi-occluded	0.5	Slightly Irritating
9	Swedish Biofuel	Occluded	0.6	Slightly Irritating
		Semi-occluded	0.6	Slightly Irritating
10	HEFA-A (Syntroleum Sapphire)	Occluded	0.6	Slightly Irritating
		Semi-occluded	0.1	Slightly Irritating

4.3 Compliance, Quality Assurance and Data Retention

The required compliance and quality assurance statements for Good Laboratory Practices (GLP) can be found in Appendix F. The U.S. Air Force, through the Henry M. Jackson Foundation for the Advancement of Military Medicine, has title to all documentation records, raw data, specimens, or other work product generated during the performance of the study. All remaining work products generated by WIL, including raw paper data and specimens, are retained in the WIL Archives as specified in the study protocol. Reserve samples of the test substances, pertinent electronic storage media, and the original final report are retained in the WIL Archives in compliance with regulatory requirements.

5.0 DISCUSSION AND CONCLUSIONS

There were no deaths or remarkable body weight changes noted during this dermal irritation study in New Zealand white rabbits. Dermal findings during the study consisted of very slight to slight erythema, as well as very slight edema. A score of slightly irritating, as evaluated by the PDII and descriptive rating, was determined for the occluded and semi-occluded exposures to all test substances, with the exception of a score of non-irritating for the semi-occluded exposures of Sasol IPK, Syntroleum S-8 and HEFA-F. Very slight erythema persisted through study day 14 for the occluded exposures to Amyris IPK, Swedish Biofuel, and HEFA-A.

As mentioned above, petroleum-derived JP-8 (POSF 4658) ranged from slightly to moderately irritating when tested previously in this assay (Hurley *et al.*, 2011; Mattie *et al.*, 2013). As expected, JP-8 (POSF 4658) was again found to be slightly irritating. Irritation did not persist beyond the third or fourth day following JP-8 exposure in any of these three assays.

One fuel produced by the FT-SPK synthesis process has been tested for dermal irritation potential previously. Hurley *et al.* (2011) found a previous batch of Syntroleum S-8 (POSF 5109) to be moderately irritating in the occluded exposure and slightly irritating in the semi-occluded exposure. Exposure to a 50:50 blend of S-8 and JP-8 (POSFs 5109 and 4658) resulted in lower dermal irritation scores than either fuel alone. In contrast, the later batch of Syntroleum S-8 (POSF 4734) tested herein was slightly irritating when occluded and non-irritating when semi-occluded. All of the SPK fuels tested herein were found to be only slightly irritating in comparison. Sasol IPK was also non-irritating in the semi-occluded exposures.

Several renewable bio-based HEFA fuels (formerly known as HRJ fuels) have been evaluated for dermal irritation previously. Mattie *et al.* (2013) tested fuels from three different HEFA feedstocks: animal fat (tallow, HEFA-T, POSF 6308), mixed fats and oils (HEFA-F, POSF 5469), and plant oil (camelina plant (*Camelina sativa*), HEFA-C, POSF 6152). All three HEFA fuels were slightly irritating in both occluded and semi-occluded exposures; HEFA-F was non-irritating in the semi-occluded assay (Mattie *et al.*, 2013). The HEFA-F tested herein is identical to the HEFA-F tested previously, with identical results, indicating reproducibility between assays.

In general, the bio-based alternative fuels appeared to be more irritating than the SPK fuels. Only HEFA-F was found to be non-irritating in the semi-occluded exposure. Further, three bio-based fuels (Amyris IPK, Swedish Biofuel, and HEFA-A) resulted in an extended period of erythema, even though the PDII scores were not higher than any of the other fuels during the first 72 hours.

In conclusion, the nine alternative fuels tested in a dermal irritation assay in New Zealand white rabbits were found to be equivalent to or less irritating than petroleum-derived JP-8. Based on this assay, it is not expected that normal handling of these fuels would result in increased dermal irritation among airmen.

6.0 REFERENCES

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APPENDIX A. STUDY PROTOCOL

PROTOCOL

ACUTE DERMAL IRRITATION STUDY OF ALTERNATIVE AND BIO-BASED JET FUELS IN NEW ZEALAND WHITE RABBITS

Submitted To:

The Henry M. Jackson Foundation
for the Advancement of Military Medicine
1401 Rockville Pike, Suite 600
Rockville, MD 20852

WIL Research Laboratories, LLC
1407 George Road
Ashland, OH 44805-8946

1 OBJECTIVE:

To determine the irritative potential of the test articles following a single exposure to the skin of albino rabbits.

This protocol has been designed and the study will be conducted in general compliance with the following guidelines:

Environmental Protection Agency (EPA) Office of Prevention, Pesticides and Toxic Substance (OPPTS) guideline 870.2500 (1998).

Organisation for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals, Section 404 (2002).

The European Union (EU) Guideline in the Official Journal of the European Communities [92/69, Annex V, B4 (1992)l.

The study will be conducted in compliance with the U.S. EPA Good Laboratory Practices (40 CFR Part 792), with the exception that analytical confirmation of the concentration, homogeneity and stability of the dosing mixture (if prepared) will not be performed.

2 PERSONNEL INVOLVED IN THE STUDY:

2.1 Sponsor Representative:

David R. Mattie, PhD, DABT
711 HPW/RHPB
2729 R Street, Bldg 837
Wright-Patterson AFB, OH 45433-5707
Phone: (937) 904-9569
Email: David.Mattie@WPAFB.AF.MIL

2.2 WIL Study Director:

Jonathan M. Hurley, BS
Project Specialist, General Toxicology
Phone: (419) 289-8700
Fax: (419) 289-3650
E-mail: jhurley@wilresearch.com

2.3 WIL Departmental Responsibilities:

Teresa D. Morris, BS
Senior Operations Manager, General Toxicology
Emergency Contact
Phone: (419) 289-8700
E-mail: tmorris@wilresearch.com

Mark D. Nemec, BS, DABT
President and Chief Operating Officer

Christopher P. Chengelis, PhD, DABT
Vice President and Chief Scientific Officer

Jozef J.W.M. Mertens, PhD, DABT
Director, General Toxicology

Eric L. Padgett, PhD
Associate Director, Toxicology and
Head of Juvenile Toxicology

Ronald E. Wilson, BS
Director, Informational Systems

Sally A. Keets, AS
Senior Operations Manager, Vivarium

Theresa M. Rafeld
Group Manager, Formulations Laboratory

Walter R. Miller, Jr., BS, DVM
Clinical Veterinarian and Head of Surgery
and Experimental Medicine

Robert A. Wally, BS, RAC
Manager, Reporting
and Regulatory Technical Services

Heather L. Johnson, BS, RQAP-GLP
Manager, Quality Assurance

3 STUDY SCHEDULE:

Proposed Experimental Start Date: May 13, 2010

Proposed Experimental Termination Date: May 27, 2010

Proposed Audited Draft Report Date: July 8, 2010

4 TEST ARTICLES:

The Sponsor assumes responsibility for purity and stability determinations (including under test conditions). Information on composition and method of synthesis will be held by the Sponsor.

4.1 Test Article #1 Identification I Lot Number:

JP-8 / POSF4658

4.2 Test Article #2 Identification I Lot Number:

Sasol/IPK / POSF5642

4.3 Test Article #3 Identification I Lot Number:

Sasol/GTL-1 / POSF5976

4.4 Test Article #4 Identification / Lot Number:

Sasol/GTL-2 / POSF5977

4.5 Test Article #5 Identification / Lot Number:

Shell GTL / POSF5 172

4.6 Test Article #6 Identification I Lot Number:

S-8 / POSF4734

4.7 Test Article #7 Identification I Lot Number:

R-8 / POSF5469

4.8 Test Article #8 Identification I Lot Number:

Amyris / POSF5630

4.9 Test Article #9 Identification I Lot Number:

Swedish Biofuel / POSF5668

4.10 Test Article #10 Identification I Lot Number:

R-8 from Algae (Syntroleum/Sapphire) / POSF5804

4.11 Purity:

Reported by Sponsor to be greater than 99 percent

4.12 Stability:

Reported by Sponsor to be stable for years when properly stored; except, Sasol/IPK (Test Article #2) assigned an expiration date of one year from date of receipt.

4.13 Physical Descriptions:

To be documented by WIL Research Laboratories, LLC

4.14 Storage Conditions:

Store at room temperature. Keep containers closed tightly. Use and store these materials in cool, dry, well-ventilated areas away from heat, direct sunlight, hot metal surfaces and all sources of ignition.

4.15 Personnel Safety:

At minimum, appropriate gloves, eye protection and long sleeves (lab coat) are to be worn during dose administration. Refer to Material Safety Data Sheets for complete available information.

4.16 Retention Samples:

Retention samples of the test articles (as received) will be collected in accordance with WIL Research Laboratories, LLC SOP No. T2-001.

4.17 Unused Test Articles:

Unused portions of the test articles will be returned following the issuance of the final study report to the contact below.

David R. Mattie, PhD, DABT
711 HPW/RHPB
2729 R Street, Bldg 837

Wright-Patterson AFB, OH 45433-5707
Phone: (937) 904-9569
Email: David.Mattie@WPAFB.AF.MIL

5 TEST SYSTEM:

5.1 Species:

Albino rabbit

5.2 Breed:

New Zealand White

5.3 Source:

Covance Research Products, Inc.
(Documentation of the specific breeding facility will be maintained in the study records and included in the final report.)

5.4 Number on Stud:

Twelve animals from the acute stock colony
Males and/or females (females will be nulliparous and nonpregnant)

5.6 Body Weight Range:

2.0 kg or greater

5.7 Approximate Age:

Young adult, at least 12 weeks old at initiation of dosing

5.8 Identification System:

Each animal will be uniquely identified by a plastic ear tag displaying the animal number. Individual cage cards will be affixed to each cage and will display the animal number, group and study number.

5.9 Justification for Selection:

This species and breed is generally recognized as appropriate for acute dermal irritation studies. The number of animals selected is the minimum required to satisfy regulatory guidelines. The experimental design uses the procedures and standards required by the current federal and international regulations.

6 SPECIFIC MAINTENANCE SCHEDULE:

6.1 Animal Housing:

The animals will be housed individually in stainless steel cages in an environmentally controlled room. Animals will be housed in clean cages elevated above ground corncob bedding or other suitable material that will be changed at least twice each week. Animals will be changed out into clean cages approximately every two weeks. The facilities at WIL Research Laboratories, LLC are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

6.2 Environmental Conditions:

Controls will be set to maintain the temperature at 66 ± 5°F (19 ± 3°C) and the relative humidity at 50 ± 20 percent. Temperature .and relative humidity will be monitored continuously. Data for these two parameters will be scheduled for automatic collection on an hourly basis. Fluorescent lighting controlled by light timers will provide illumination for a 12-hour light/dark photoperiod. Temporary adjustments to the light/dark cycles may be made to accommodate protocol specified activities. The ventilation rate will be set at a minimum of 10 room air changes per hour, 100 percent fresh air.

6.3 Drinking Water:

Municipal water will be available *ad libitum*. Filters servicing the automatic watering system will be changed regularly according to Standard Operating Procedures (SOPs). Municipal water supplying the laboratory is analyzed for contaminants according to SOPs to ascertain that none are present at concentrations that would be expected to affect the outcome of the study and the results are maintained on file.

6.4 Basal Diet:

PMI Nutrition International, LLC Certified High Fiber Rabbit LabDiet® 5325 will be offered at approximately 150 g/day during the study. The amount of feed provided will be an estimate and will not be documented. Standard Operating Procedures provide specifications for acceptable levels of heavy metals and pesticides that are reasonably expected to be present in the diet without interfering with the purpose or conduct of the study. Analyses are performed and provided by the manufacturer and the results are maintained on file.

7 EXPERIMENTAL DESIGN:

7.1 Animal Receipt and Acclimation:

Each animal was/will be inspected by a qualified technician upon receipt into the acute stock colony. Animals judged to be in good health and suitable as test animals were/will be acclimated to laboratory conditions for a minimum of five days. All animals were/will be weighed initially and permanently identified.

During the acclimation period, each animal will be observed twice daily for changes in general appearance and behavior.

All relevant records and data collected during the acclimation period for animals used on this study will be maintained on file.

7.2 Veterinary Care:

Animals will be monitored by the technical staff for any condition requiring possible veterinary care. If any such condition is identified, a staff veterinarian will be notified for an examination and evaluation. Animals will be treated as outlined in the Animal Welfare Act Compliance section of the protocol.

7.3 Route and Rationale of Test Article Administration:

The route of administration will be dermal (clipped, intact skin) in order to evaluate the dermal irritation potential of the test articles. This study is intended to provide information on the health hazards likely to arise from a short-term exposure to the test articles by the dermal route.

7.4 Organization of Treatment Groups:

Following the acclimation period, animals will be arbitrarily selected from available stock based upon health and body weight and assigned to 4 groups of 3 rabbits/group as shown below. No separate control group will be utilized; each animal will serve as its own control. The skin of all test sites will be left intact (unabraded).

Group Number	Test Articles	Dose Volume (mL/Test Article)	Exposure Method	Number of Animals
1	#1, #2, #3, #4, #5	0.5	Occluded	3
2	#1, #2, #3, #4, #5	0.5	Semi-occluded	3
3	#6, #7, #8, #9, #10	0.5	Occluded	3
4	#6, #7, #8, #9, #10	0.5	Semi-occluded	3

#1 = JP-8, #2 = Sasol/IPK, #3 = Sasol/GTL-1, #4 = Sasol/GTL-2, #5 = Shell GTL,
#6 = S-8 / POSF4734, #7 = R-8, #8 = Amyris, #9 = Swedish Biofuel, #10 = R-8 from Algae

7.5 Test Material Preparation:

The test articles will be administered undiluted as received at a dosage of 0.5 mL. The pH will be determined and recorded.

7.6 Animal Preparation

On the day prior to dermal applications, the back and flanks of each animal will be clipped free of hair with a small animal clipper. The clipped area on each animal will constitute approximately 20-25 percent of the total body surface area (actual size of area will not be recorded). Animals with dermal abnormalities or injuries will be excluded.

7.7 Method of Administration

Five sites located lateral to the midline of the back will be selected on each rabbit. The location of the test sites (designated A-F based upon six available site locations on the back of the rabbit) will be rotated so that no test article is applied to the same site within a group of rabbits. The test sites will be delineated with four dots made with indelible ink spaced approximately 2.5 centimeters apart arranged in a square. All animals will receive a single application of five test articles.

Each test site will be immediately covered with a two ply, 2.5-cm square gauze patch. The patch will be secured in place with surgical porous tape. For animals in the occluded exposure groups the trunk of the animal will be wrapped with plastic wrap to occlude the test site. The trunk of animals in both the occluded and semi-occluded groups will then be wrapped with gauze bandaging that will be secured with several wrappings of non-irritating tape. Elizabethan collars will be applied to each animal during the exposure period to prevent ingestion of the test article and/or wrappings.

After the four hours of exposure, the bandages will be removed and residual test article cleansed from the application sites using clean, disposable paper towels moistened with deionized water (as thoroughly as possible without irritating the skin). The same towel will not be used on more than one site.

8 OBSERVATIONS:

8.1 Viability and Clinical Observations:

All animals will be observed for mortality/moribundity twice daily (morning and afternoon) for the duration of the study. Moribund animals will be removed from study and euthanized by intravenous injection of sodium pentobarbital. All animals will receive a detailed physical examination on the day of dosing.

8.2 Dermal Observations:

Approximately 30-60 minutes after test article removal, each test site will be examined and the degree of erythema and edema recorded according to the Draize technique (Appendix). The presence of any other dermal findings will also be recorded. Additional examinations will be performed at approximately 24, 48 and 72 hours after patch removal. If no irritation is present at the 72-hour observation, the study may be terminated.

If irritation is present at the end of 72 hours, additional observations will be performed on days 4, 7 and 14, or until irritation subsides. The study need not normally exceed 14 days after application unless specifically requested and authorized by the Sponsor. Individual animals will be terminated if no irritation is present at the 72-hour or any subsequent observation. At the request of the Sponsor, observations may be terminated prior to 14 days and/or resolution of irritation.

The areas of application will be clipped free of hair a minimum of one hour before scoring, as needed during the study, to facilitate accurate dermal observations.

8.3 Body Weights:

The body weight of each animal will be determined on study day 0 and at termination.

8.4 Gross Pathology:

All animals will be euthanized by intravenous injection of sodium pentobarbital. A gross necropsy examination on major organ systems of the thoracic and visceral cavities will be conducted on all animals found dead or euthanized *in extremis*. Animals euthanized following study termination will be discarded without further examination.

9 CALCULATION OF THE PRIMARY DERMAL IRRITATION INDEX:

The Primary Dermal Irritation Index will be calculated from the scores recorded at 30-60 minutes, 24, 48 and 72 hours (after patch removal). The mean scores for erythema and edema will be calculated separately to the nearest tenth and added together. Based on this value, the grading system in the Appendix will be used to arrive at a primary dermal irritation descriptive rating for each test article for the occluded and unoccluded method of exposure.

10 REPORT:

The final report will include, but will not necessarily be limited to, the following: compliance statement, summary, objective, test article identification and receipt information, methods, observations, mortality, body weights, individual and summarized

dermal scores/findings, classification of the test articles based on their dermal irritation properties, results and discussion, key personnel, a signed QAU statement and protocol deviation(s), if any.

WIL Research Laboratories will submit one electronic copy (PDF with an MS Word copy of the report text for editing and comments) of an audited draft report in a timely manner upon completion of data collection prior to issuance of the final report. It is expected that the Sponsor will review the draft report and provide comments to WIL within a two-month time frame following submission. Within one month following receipt of the Sponsor's comments, WIL shall provide a revised draft report that incorporates the Sponsor's reasonable revisions and suggestions. One revision will be permitted as part of the cost of the study; additional changes or revisions may be made, at extra cost. WIL will submit the final report within two weeks of receiving authorization from the Sponsor. If the Sponsor's comments and/or authorization to finalize the report have not been received at WIL within one year following submission of the draft report, WIL may elect to finalize the report following appropriate written notification to the Sponsor. Two electronic copies (PDF) of the final report on CD-R will be provided. Requests for additional paper copies of the final report may result in additional charges.

11 RECORDS TO BE MAINTAINED:

All original raw data records (as defined by the applicable GLPs and WIL SOPS) generated by WIL Research Laboratories, LLC will be collected and maintained by WIL Research Laboratories, LLC.

12 WORK PRODUCT:

Sponsor will have title to all documentation records, raw data, slides, specimens, or other work product generated during the performance of the study. All work product including raw paper data, pertinent electronic storage media and specimens will be retained at no charge for a period of six months following issuance of the final report in the Archives at WIL Research Laboratories, LLC. Thereafter, WIL Research Laboratories will charge a monthly archiving fee for retention of all work products. All work products will be stored in compliance with regulatory requirements.

Any work product, including documents, specimens, and samples, that are required by this protocol, its amendments, or other written instructions of the Sponsor, to be shipped by WIL Research Laboratories, LLC to another location will be appropriately packaged and labeled as defined by WIL's SOPs and delivered to a common carrier for shipment. WIL Research Laboratories, LLC will not be responsible for shipment following delivery to the common carrier.

13 QUALITY ASSURANCE:

The study will be audited by the WIL Quality Assurance Unit while in progress to assure compliance with EPA Good Laboratory Practices and adherence to the protocol and to WIL SOPs. The raw data and. draft report will be audited by the WIL Quality Assurance Unit to assure that the final report accurately describes the conduct and the findings of the study.

14 PROTOCOL MODIFICATION:

Modification of the protocol may be accomplished during the course of this investigation. However, no changes will be made in the study design without the verbal or written permission of the Sponsor. In the event that the Sponsor verbally requests or approves changes in the protocol, such changes will be made by appropriate documentation in the form of protocol amendments. All alterations of the protocol and reasons for the modification(s) will be signed by the Study Director and the Sponsor Representative.

15 ANIMAL WELFARE ACT COMPLIANCE:

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR). The Sponsor should make particular note of the following:

- The Sponsor signature on this protocol documents for the Study Director the Sponsor's assurance that, for the study described in this protocol, there are no acceptable non-animal alternatives and the study does not unnecessarily duplicate previous experiments.
- Whenever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory SOPS.
- Animals that experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized as deemed appropriate by the veterinary staff and Study Director. The Sponsor will be advised by the Study Director of all circumstances which could lead to this action in as timely a manner as possible.
- Methods of euthanasia used during this study are in conformance with the above-referenced regulation.
- The Sponsor/Study Director has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals and has provided a written narrative description (AWA covered species) of the methods and sources used to determine that alternatives are not available.

16 PROTOCOL APPROVAL:

Sponsor approval received by the Study Director via email on April 23, 2010.

The Henry M. Jackson Foundation for the Advancement of Military Medicine

David R. Mattie, PhD, DABT
Sponsor Representative

4 May 10
Date

WIL Research Laboratories, LLC

Jonathan M. Hurley, BS
Study Director

4/30/10
Date

APPENDIX

SCORING CRITERIA FOR DERMAL REACTIONS*

Value	Erythema and Eschar Formation
0	No erythema
1	Very slight erythema (barely perceptible, edges of area not well defined)
2	Slight erythema (pale red in color and edges definable)
3	Moderate to severe erythema (definite red in color and area well defined)
4	Severe erythema (beet or crimson red) to slight eschar formation (injuries in depth)
4	Maximum possible erythema score

	Edema Formation
0	No edema
1	Very slight edema (barely perceptible, edges of area not well defined)
2	Slight edema (edges of area well defined by definite raising)
3	Moderate edema (raised approximately 1 mm)
4	Severe edema (raised more than 1 mm and extending beyond area of exposure)
4	Maximum possible edema score
8	Maximum total possible Primary Irritation Score

DESCRIPTIVE RATINGS

Mean Primary Dermal Irritation Index

Range of Values	Descriptive Rating
0	Nonirritating
0.1 - 2.0	Slightly Irritating
2.1 - 5.0	Moderately Irritating
5.1 - 8.0	Severely Irritating

*Draize, J.H., 1965. The Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Dermal Toxicity, pp. 46-59. Assoc. of Food and Drug Officials of the U.S., Topeka, Kansas and the EPA-OPPTS Health Effects Test Guidelines (1998).

APPENDIX B. SCORING CRITERIA FOR DERMAL REACTIONS

Evaluation of Dermal Reactions[a]

Value	Erythema and Eschar Formation
0	No erythema
1	Very slight erythema (barely perceptible, edges of area not well defined)
2	Slight erythema (pale red in color and edges definable)
3	Moderate to severe erythema (definite red in color and area well defined)
4	Severe erythema (beet or crimson red) to slight eschar formation (injuries in depth)
4	Maximum possible erythema score

Value	Edema Formation
0	No edema
1	Very slight edema (barely perceptible, edges of area not well defined)
2	Slight edema (edges of area well defined by definite raising)
3	Moderate edema (raised approximately 1 mm)
4	Severe edema (raised more than 1 mm and extending beyond area of exposure)
4	Maximum possible edema score

8 Maximum total possible Primary Irritation Score

Descriptive Ratings

Mean Primary Dermal Irritation Index Range of Values	Descriptive Rating
0	Nonirritating
0.1 - 2.0	Slightly Irritating
2.1 - 5.0	Moderately Irritating
5.1 - 8.0	Severely Irritating

[a]Draize, J.H., 1965. The Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Dermal Toxicity, pp. 46-59. Assoc. of Food and Drug Officials of the U.S., Topeka, Kansas and the EPA-OPPTS Health Effects Test Guidelines (1998).

APPENDIX C. DATA ACQUISITION AND REPORTING SYSTEMS

Program/System	Description
Archive Management System (AMS)	In-house developed application for storage, maintenance, and retrieval information for archived materials (*e.g.*, lab books, study data, wet tissues, slides, etc.)
Formulations Dose Dispensing Management System (FDDMS)	In-house developed system used to assign unique barcodes to formulation containers and individual containers used for dispensing dosing formulations.
InSight® Publisher	Electronic publishing system (output is Adobe Acrobat, PDF)
Master Schedule	Maintains the master schedule for the company.
Metasys DDC Electronic Environmental Control System	Controls and monitors animal room environmental conditions.
Microsoft® Office 2002 and 2007; GraphPad Prism® 2008	Used in conjunction with the publishing software to generate study reports.
Provantis Dispense™	Comprehensive system (Instem LSS Limited) to manage test materials, including receipt, formulation instructions, and accountability.
WIL Metasys	In-house developed system used to record and report animal room environmental conditions.
WIL Formulations Dispense System (WFDS)	In-house developed system for use in conjunction with Provantis Dispense™ to ensure proper storage and use of formulations.

APPENDIX D. ANIMAL ROOM ENVIRONMENTAL CONDITIONS

STUDY SPECIFICATIONS: 773001 DATE IN 05/13/10 TIME IN 07:00
DATE OUT 05/28/10 TIME OUT 14:00

ROOM SPECIFICATIONS: B ROOM 57 LOW TEMPERATURE °F: 61.0 HIGH TEMPERATURE °F: 71.0 LOW HUMIDITY %RH: 30.0

TEST SYSTEM: RABBIT LOW TEMPERATURE °C: 16.1 HIGH TEMPERATURE °C: 21.7 HIGH HUMIDITY %RH: 70.0

	PRIMARY TEMP		SECONDARY TEMP		PRIMARY HUM	SECONDARY HUM
DATE	MEAN (°F)	MEAN (°C)	MEAN (°F)	MEAN (°C)	MEAN (%RH)	MEAN (%RH)
05/13/10	67.2	19.6	68.1	20.1	54.4	55.3
05/14/10	66.4	19.1	67.5	19.7	52.8	53.5
05/15/10	66.9	19.4	68.0	20.0	50.2	51.0
05/16/10	66.4	19.1	67.7	19.8	51.1	52.0
05/17/10	66.0	18.9	67.5	19.7	52.9	53.8
05/18/10	66.4	19.1	67.8	19.9	52.5	53.2
05/19/10	66.1	18.9	67.4	19.7	52.7	53.7
05/20/10	66.5	19.2	67.6	19.8	51.0	52.1
05/21/10	66.8	19.3	68.0	20.0	52.2	52.7
05/22/10	66.7	19.3	67.9	19.9	52.7	53.2
05/23/10	66.6	19.2	67.7	19.8	52.9	53.6
05/24/10	66.7	19.3	67.8	19.9	53.6	54.2
05/25/10	66.8	19.3	67.7	19.8	54.2	54.9
05/26/10	67.0	19.4	67.9	19.9	55.4	56.0
05/27/10	66.6	19.2	67.6	19.8	52.4	52.9
05/28/10	66.7	19.3	67.8	19.9	51.0	51.3

DATE	PRIMARY TEMP MEAN (°F)	PRIMARY TEMP MEAN (°C)	SECONDARY TEMP MEAN (°F)	SECONDARY TEMP MEAN (°C)	PRIMARY HUM MEAN (%RH)	SECONDARY HUM MEAN (%RH)
SUMMARY OF DAILY MEANS	MEAN	MIN	MAX			
PRIMARY TEMP °F:	66.6	66.0	67.2			
PRIMARY TEMP °C:	19.2	18.9	19.6			
SECONDARY TEMP °F:	67.7	67.4	68.1			
SECONDARY TEMP °C:	19.8	19.7	20.1			
PRIMARY HUM %RH:	52.6	50.2	55.4			
SECONDARY HUM %RH:	53.4	51.0	56.0			
N DAYS	16					

B ROOM 57 SUMMARY OF HOURLY VALUES

	PRIMARY TEMP		SECONDARY TEMP		PRIMARY HUM	SECONDARY HUM
MEAN	66.6 °F	19.2 °C	67.7 °F	19.8 °C	52.6 %RH	53.4 %RH
MIN	64.8 °F	18.2 °C	66.4 °F	19.1 °C	48.0 %RH	48.2 %RH
MAX	70.7 °F	21.5 °C	70.3 °F	21.3 °C	68.9 %RH	70.0 %RH
SD	0.83	0.46	0.64	0.36	2.25	2.42
SE	0.04	0.02	0.03	0.02	0.12	0.13
N SAMPLES	364		364		364	364
FIRST DAY	05/13/10					
LAST DAY	05/28/10					
N DAYS	16					

STUDY 773001 SUMMARY OF HOURLY VALUES

	PRIMARY TEMP		SECONDARY TEMP		PRIMARY HUM	SECONDARY HUM
MEAN	66.6 °F	19.2 °C	67.7 °F	19.8 °C	52.6 %RH	53.4 %RH
MIN	64.8 °F	18.2 °C	66.4 °F	19.1 °C	48.0 %RH	48.2 %RH
MAX	70.7 °F	21.5 °C	70.3 °F	21.3 °C	68.9 %RH	70.0 %RH
SD	0.83	0.46	0.64	0.36	2.25	2.42
SE	0.04	0.02	0.03	0.02	0.12	0.13
N SAMPLES	364		364		364	364
FIRST DAY	05/13/10					
LAST DAY	05/28/10					
N DAYS	16					

APPENDIX E. INDIVIDUAL DERMAL DATA

Material: JP-8, Occluded

Site: 0.5 mL/Site

Animal	Sex	Site	Erythema							Edema					
			0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60638	M	A	1	0	1	1	1	0	0	0	0	0	0	0	0	0
60639	M	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60640	M	E	1	0	0	0	0	NA	NA	0	0	0	0	0	NA	NA

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (2 + 0 + 1 + 1) / 12 + (0 + 0 + 0 + 0) / 12

PII = 4 / 12 + 0 / 12

PII = 0.3+ 0.0

PII = 0.3 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations: Head

A D

B E

C F

Tail

Material: JP-8, Semi-occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60634	M	A	0	1	1	1	1	0	NA	0	0	0	0	0	0	NA
60636	M	C	1	0	1	0	NA	NA	NA	0	0	0	0	NA	NA	NA
60637	M	E	0	0	1	1	1	0	NA	0	0	0	0	0	0	NA

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = $(1 + 1 + 3 + 2) / 12 + (0 + 0 + 0 + 0) / 12$

PII = $7 / 12 + 0 / 12$

PII = $0.6 + 0.0$

PII = 0.6 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: Sasol/IPK, Occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60638	M	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60639	M	D	1	0	1	1	1	0	0	0	0	0	0	0	0	0
60640	M	F	0	0	0	0	0	NA	NA	0	0	0	0	0	NA	NA

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (1 + 0 + 1 + 1) / 12 + (0 + 0 + 0 + 0) / 12

PII = 3 / 12 + 0 / 12

PII = 0.3 + 0.0

PII = 0.3 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: Sasol/IPK, Semi-occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60634	M	B	0	0	0	0	0	0	NA	0	0	0	0	0	0	NA
60636	M	D	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA
60637	M	F	0	0	0	0	0	0	NA	0	0	0	0	0	0	NA

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (0 + 0 + 0 + 0) / 12 + (0 + 0 + 0 + 0) / 12

PII = 0 / 12 + 0 / 12

PII = 0.0 + 0.0

PII = 0.0 = Nonirritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: Sasol/GTL-1, Occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60638	M	C	0	0	0	0	1	0	0	0	0	0	0	0	0	0
60639	M	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60640	M	A	1	0	1	1	0	NA	NA	0	0	0	0	0	NA	NA

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (1 + 0 + 1 + 1) / 12 + (0 + 0 + 0 + 0) / 12

PII = 3 / 12 + 0 / 12

PII = 0.3 + 0.0

PII = 0.3 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: Sasol/GTL-1, Semi-occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60634	M	C	0	0	1	1	0	0	NA	0	0	0	0	0	0	NA
60636	M	E	0	1	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA
60637	M	A	0	0	0	0	0	0	NA	0	0	0	0	0	0	NA

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (0 + 1 + 1 + 1) / 12 + (0 + 0 + 0 + 0) / 12

PII = 3 / 12 + 0 / 12

PII = 0.3 + 0.0

PII = 0.3 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: Sasol/GTL-2, Occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60638	M	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60639	M	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60640	M	B	1	0	0	0	0	NA	NA	0	0	0	0	0	NA	NA

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (1 + 0 + 0 + 0) / 12 + (0 + 0 + 0 + 0) / 12

PII = 1 / 12 + 0 / 12

PII = 0.1 + 0.0

PII = 0.1 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: Sasol/GTL-2, Semi-occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60634	M	D	0	1	0	0	0	0	NA	0	0	0	0	0	0	NA
60636	M	F	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA
60637	M	B	0	0	0	0	0	0	NA	0	0	0	0	0	0	NA

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = $(0 + 1 + 0 + 0) / 12 + (0 + 0 + 0 + 0) / 12$

PII = $1 / 12 + 0 / 12$

PII = $0.1 + 0.0$

PII = 0.1 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: Shell GTL, Occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60638	M	E	0	0	1	1	1	1	0	0	0	0	0	0	0	0
60639	M	A	0	0	0	0	1	1	0	0	0	0	0	0	0	0
60640	M	C	0	0	0	0	0	NA	NA	0	0	0	0	0	NA	NA

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = $(0 + 0 + 1 + 1) / 12 + (0 + 0 + 0 + 0) / 12$

PII = $2 / 12 + 0 / 12$

PII = $0.2 + 0.0$

PII = 0.2 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: Shell GTL, Semi-occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60634	M	E	0	0	0	1	1	0	NA	0	0	0	0	0	0	NA
60636	M	A	0	1	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA
60637	M	C	0	0	0	0	0	0	NA	0	0	0	0	0	0	NA

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (0 + 1 + 0 + 1) / 12 + (0 + 0 + 0 + 0) / 12

PII = 2 / 12 + 0 / 12

PII = 0.2 + 0.0

PII = 0.2 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: S-8, Occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60630	M	A	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA
60632	M	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60633	M	E	1	1	1	0	0	0	0	0	0	0	0	0	0	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (1 + 1 + 1 + 0) / 12 + (0 + 0 + 0 + 0) / 12

PII = 3 / 12 + 0 / 12

PII = 0.3 + 0.0

PII = 0.3 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: S-8, Semi-occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60625	M	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60626	M	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60629	M	E	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (0 + 0 + 0 + 0) / 12 + (0 + 0 + 0 + 0) / 12

PII = 0 / 12 + 0 / 12

PII = 0.0 + 0.0

PII = 0.0 = Nonirritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: R-8, Occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60630	M	B	1	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA
60632	M	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60633	M	F	1	1	0	0	0	0	0	0	0	0	0	0	0	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (2 + 1 + 0 + 0) / 12 + (0 + 0 + 0 + 0) / 12

PII = 3 / 12 + 0 / 12

PII = 0.3 + 0.0

PII = 0.3 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: R-8, Semi-occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60625	M	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60626	M	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60629	M	F	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (0 + 0 + 0 + 0) / 12 + (0 + 0 + 0 + 0) / 12

PII = 0 / 12 + 0 / 12

PII = 0.0 + 0.0

PII = 0.0 = Nonirritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: Amyris, Occluded
Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60630	M	C	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA
60632	M	E	1	0	1	1	1	1	1	0	0	0	0	0	0	0
60633	M	A	0	2	1	1	0	1	0	0	1	0	0	0	1	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = $(1+2+2+2)/12 + (0+1+0+0)/12$

PII = $7/12 + 1/12$

PII = $0.6 + 0.1$

PII = 0.7 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: Amyris, Semi-occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60625	M	C	0	1	1	1	0	0	0	0	0	0	0	0	0	0
60626	M	E	0	1	1	1	1	1	0	0	0	0	0	0	0	0
60629	M	A	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (0 + 2 + 2 + 2) / 12 + (0 + 0 + 0 + 0) / 12

PII = 6 / 12 + 0 / 12

PII = 0.5 + 0.0

PII = 0.5 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: Swedish Biofuel, Occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60630	M	D	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA
60632	M	F	1	0	1	1	1	1	1	0	0	0	0	0	0	0
60633	M	B	1	1	1	1	1	1	0	0	0	0	0	0	0	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (2 + 1 + 2 + 2) / 12 + (0 + 0 + 0 + 0) / 12

PII = 7 / 12 + 0 / 12

PII = 0.6 + 0.0

PII = 0.6 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: Swedish Biofuel, Semi-occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60625	M	D	0	1	1	1	2	1	0	0	0	1	1	1	0	0
60626	M	F	0	1	0	1	0	0	0	0	0	0	0	0	0	0
60629	M	B	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = $(0 + 2 + 1 + 2) / 12 + (0 + 0 + 1 + 1) / 12$

PII = $5 / 12 + 2 / 12$

PII = $0.4 + 0.2$

PII = 0.6 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: R-8 from Algae (Syntroleum/Sapphire), Occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60630	M	E	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA
60632	M	A	1	1	1	1	1	1	1	0	0	0	0	0	0	0
60633	M	C	0	1	1	1	0	0	0	0	0	0	0	0	0	0

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = $(1+2+2+2)/12 + (0+0+0+0)/12$

PII = $7/12 + 0/12$

PII = $0.6 + 0.0$

PII = 0.6 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

Material: R-8 from Algae (Syntroleum/Sapphire), Semi-occluded

Site: 0.5 mL/Site

			Erythema							Edema						
Animal	Sex	Site	0.5-1H	24H	48H	72H	4D	7D	14D	0.5-1H	24H	48H	72H	4D	7D	14D
60625	M	E	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60626	M	A	0	0	1	0	0	0	0	0	0	0	0	0	0	0
60629	M	C	0	0	0	0	NA	NA	NA	0	0	0	0	NA	NA	NA

PII Calculated Using Test Periods: 1H, 24H, 48H, 72H

Primary Irritation Index (PII) = (0 + 0 + 1 + 0) / 12 + (0 + 0 + 0 + 0) / 12

PII = 1 / 12 + 0 / 12

PII = 0.1 + 0.0

PII = 0.1 = Slightly Irritating

M = Male; H = Hours; D = Day; NA = Not Applicable

Site Locations:

Head

A D

B E

C F

Tail

APPENDIX F. COMPLIANCE AND QUALITY ASSURANCE STATEMENTS

WIL-773001 Alternative and Bio-Based Jet Fuels
The Henry M. Jackson Foundation

COMPLIANCE STATEMENT

This study, designated WIL-773001, was conducted in compliance with the United States EPA GLP Standards (40 CFR Part 792), 18 September 1989; the WIL SOPs; and the protocol as approved by the Sponsor with the following exception. Analytical confirmation of the concentration, purity, homogeneity, and stability of the test substances was not supplied by the Sponsor and was not conducted as part of this study.

The protocol was designed to be in general accordance with the EPA OPPTS guideline 870.2500 (1998), OECD Guidelines for Testing of Chemicals, Section 404 (2002), and the EU Guideline in the Official Journal of the European Communities [92/69, Annex V, B4 (1992)].

Jonathan M. Hurley, BS
Project Specialist, General Toxicology
Study Director

3 Aug 2010
Date

QUALITY ASSURANCE: PHASES INSPECTED

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management	Auditor(s)
13-May-2010, 14-May-2010	Dermal Test Substance Administration	14-May-2010	28-Jun-2010	R. Rohr
07-Jun-2010, 08-Jun-2010, 09-Jun-2010, 10-Jun-2010, 30-Jun-2010	Study Records (I-1)	30-Jun-2010	21-Jul-2010	R. Siburt
10-Jun-2010, 30-Jun-2010	Study Records (Rx-1)	30-Jun-2010	21-Jul-2010	R. Siburt
10-Jun-2010, 30-Jun-2010	Study Records (Rx-1) Supplemental	30-Jun-2010	21-Jul-2010	M. Salyers / R. Siburt
23-Jun-2010, 24-Jun-2010, 30-Jun-2010	Draft Report	30-Jun-2010	21-Jul-2010	M. Salyers / R. Siburt

This study was inspected in accordance with the United States EPA GLP Standards (40 CFR Part 792), the WIL SOPs, and the Sponsor's protocol. Quality Assurance findings, derived from the inspections during the conduct of the study and from the inspections of the raw data and draft report, are documented and have been reported to the study director. Review of the protocol and protocol amendments (if applicable) as well as a yearly internal facility inspection are conducted by the WIL Quality Assurance Unit. A status report is submitted to management monthly.

This report accurately reflects the data generated during the study. The methods and procedures used in the study were those specified in the protocol, its amendments, and the WIL SOPs.

QUALITY ASSURANCE: APPROVAL

This study was inspected according to the criteria discussed above.

Report Audited by:

Mary B. Salyers, BA
Compliance Specialist

3 Aug 2010
Date

Robyn A. Siburt, AAS, RLAT
Compliance Specialist

3 Aug 2010
Date

Report Released by:

Heather L. Johnson, BS, RQAP-GLP
Manager, Quality Assurance

3 Aug 2010
Date

LIST OF ACRONYMS

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
AFB	Air Force Base
AFRL	Air Force Research Laboratory
ATJ	alcohol to jet
AVMA	American Veterinary Medical Association
EPA	Environmental Protection Agency
FT-SPK	Fischer-Tropsch synthetic paraffinic kerosene
GLP	Good Laboratory Practices
GTL	gas to liquid
HEFA	hydroprocessed esters and fatty acids
HEFA-A	HEFA-algae
HEFA-C	HEFA-camelina
HEFA-F	HEFA-animal fats and oils
HEFA-T	HEFA-tallow
HJF	Henry M. Jackson Foundation for the Advancement of Military Medicine
HRJ	hydrotreated renewable jet
IPK	iso-paraffinic kerosene
OECD	Organisation for Economic Cooperation and Development
OPPTS	Office of Prevention, Pesticides and Toxic Substances
PDII	Primary Dermal Irritation Index
R-8	renewable JP-8
S-8	synthetic JP-8
SOP	standard operating procedure
SPK	synthetic paraffinic kerosene
SSI	Sasol Synfuels International
WIL	WIL Research Laboratories, LLC